



Investigation of genome variations during modern breeding of *Brassica napus*

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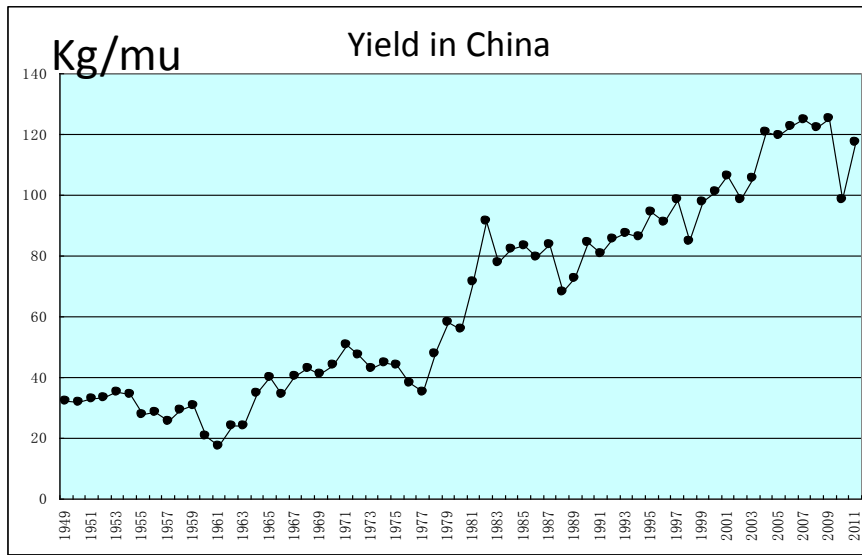
Oil Crops Research Institute, CAAS, China

April 1, Brassica 2014, Wuhan





Progress of rapeseed breeding in past 60 years



Erucic acid
Glucosinolate

High to “double low”



Oil Content
From ~30% to 50%

.....

However, it is more and more difficult to make new similar progress in rapeseed breeding now



Questions should be addressed

- ✓ What pattern of genetic changes is underlying rapeseed genome during rapeseed breeding over past decades corresponding to the breeding progress
- ✓ How to make more progress in rapeseed breeding in future

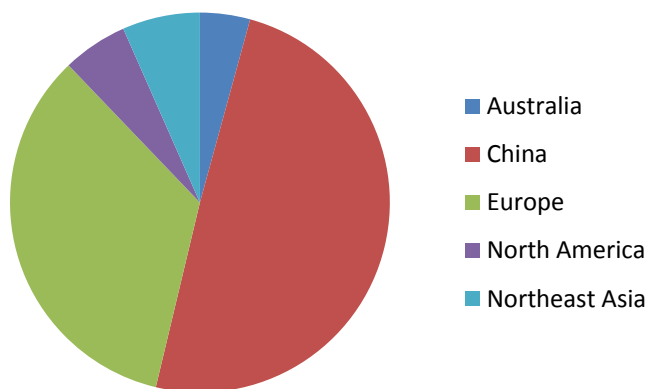


Genome-wide investigation of genetic changes during modern breeding of *Brassica napus*

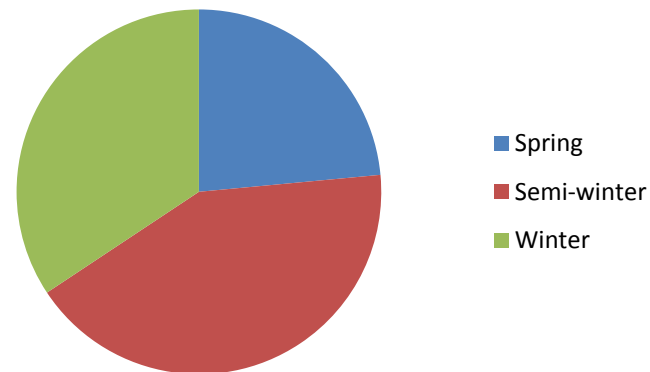


A word-wide rapeseed core collection (472 accessions)

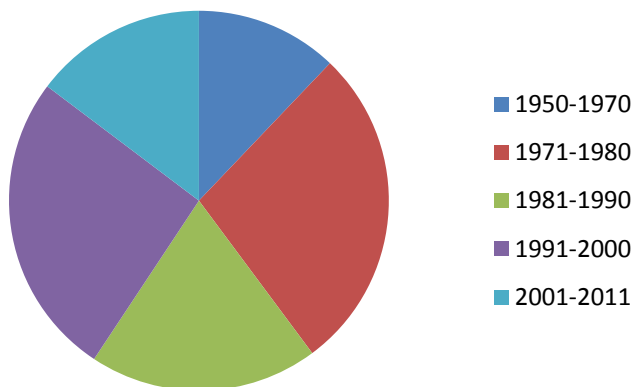
It was selected from ~8000 rapeseed germplasm according to their field performance, released time, countries and growth types.



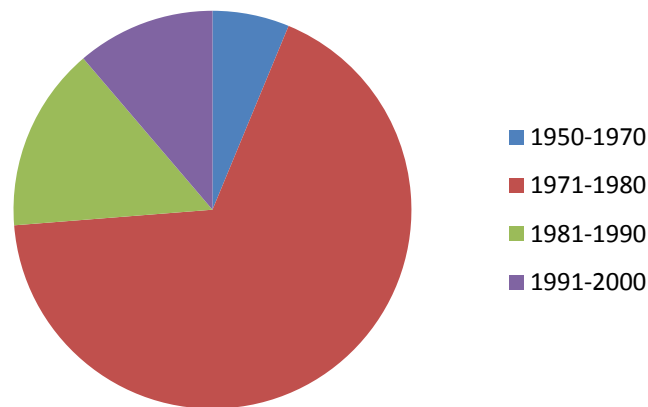
Geography



Growth Types



Release periods in China

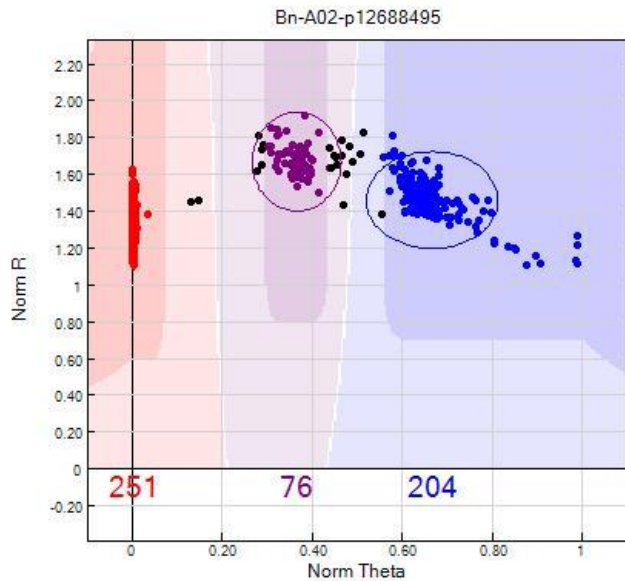


Release periods in Europe

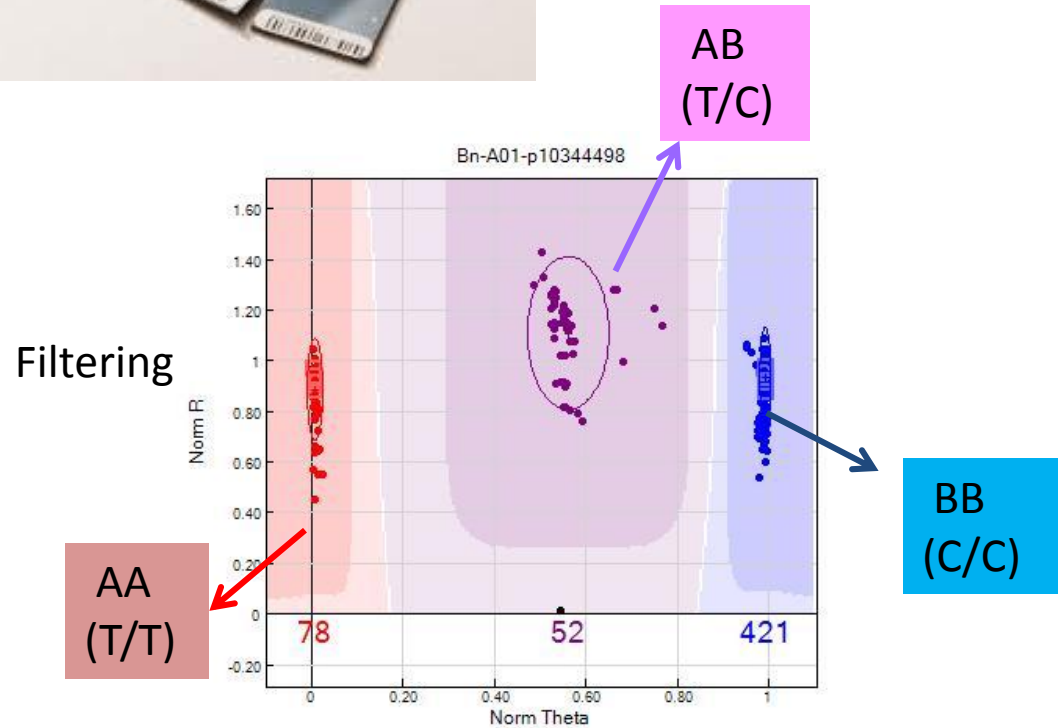
Genotyping of 472 world-wide rapeseed collection



Illumina Infinium 60k SNP chip Developed by *Brassica napus* genome sequencing project consortium



Representative SNP that failed



Representative SNP scored accurately

In silico mapping of 60 K SNPs

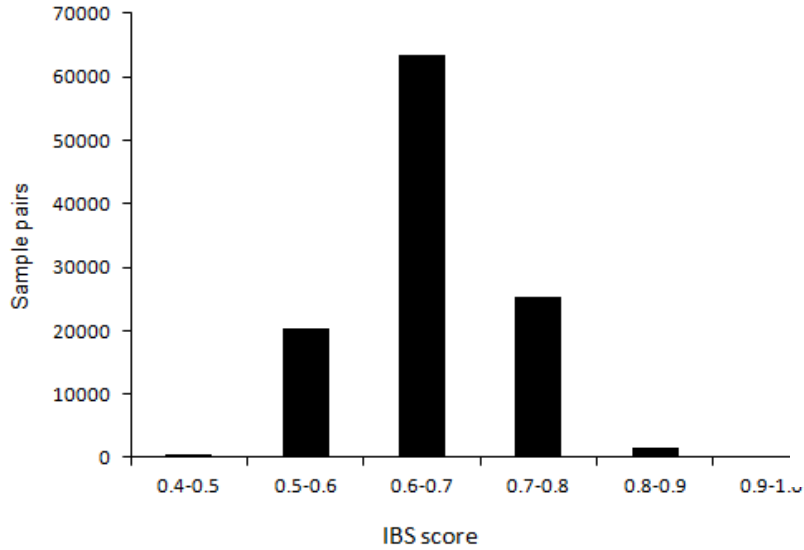
‘Pseudomolecules’ constructed by professor Ian Bancroft (Harper et al. 2012)

| Linkage group | Number of SNPs | PIC value | | | | | | | Average PIC* |
|---------------|----------------|------------|-------------|-------------|-------------|--------------|--------------|--------------|--------------|
| | | 0.05~0.1 | 0.1~0.15 | 0.15~0.2 | 0.2~0.25 | 0.25~0.3 | 0.3~0.35 | 0.35~0.4 | |
| A1 | 1072 | 9 (0.8%) | 34 (3.2%) | 83 (7.7%) | 117 (10.9%) | 102 (9.5%) | 248 (23.1%) | 479 (44.7%) | 0.31 g |
| A2 | 506 | 2 (0.4%) | 34 (6.7%) | 25 (4.9%) | 46 (9.1%) | 115 (22.7%) | 130 (25.7%) | 154 (30.4%) | 0.294 cd |
| A3 | 1469 | 6 (0.4%) | 45 (3.1%) | 93 (6.3%) | 130 (8.8%) | 210 (14.3%) | 383 (26.1%) | 602 (41%) | 0.311 g |
| A4 | 1035 | 6 (0.6%) | 61 (5.9%) | 56 (5.4%) | 131 (12.7%) | 209 (20.2%) | 272 (26.3%) | 300 (29%) | 0.293 bcd |
| A5 | 1123 | 1 (0.1%) | 35 (3.1%) | 69 (6.1%) | 115 (10.2%) | 179 (15.9%) | 376 (33.5%) | 348 (31%) | 0.307 fg |
| A6 | 1099 | 3 (0.3%) | 36 (3.3%) | 58 (5.3%) | 91 (8.3%) | 168 (15.3%) | 317 (28.8%) | 426 (38.8%) | 0.312 g |
| A7 | 1427 | 11 (0.8%) | 50 (3.5%) | 70 (4.9%) | 126 (8.8%) | 217 (15.2%) | 382 (26.8%) | 571 (40%) | 0.31 g |
| A8 | 691 | 7 (1%) | 35 (5.1%) | 38 (5.5%) | 40 (5.8%) | 53 (7.7%) | 143 (20.7%) | 375 (54.3%) | 0.319 h |
| A9 | 1225 | 9 (0.7%) | 45 (3.7%) | 39 (3.2%) | 68 (5.6%) | 190 (15.5%) | 261 (21.3%) | 613 (50%) | 0.32 h |
| A10 | 805 | 8 (1%) | 33 (4.1%) | 38 (4.7%) | 72 (8.9%) | 104 (12.9%) | 193 (24%) | 357 (44.3%) | 0.312 g |
| C1 | 2012 | 1 (0%) | 73 (3.6%) | 124 (6.2%) | 238 (11.8%) | 298 (14.8%) | 1074 (53.4%) | 204 (10.1%) | 0.298 cde |
| C2 | 1292 | 2 (0.2%) | 23 (1.8%) | 54 (4.2%) | 116 (9%) | 397 (30.7%) | 321 (24.8%) | 379 (29.3%) | 0.3 de |
| C3 | 2201 | 29 (1.3%) | 77 (3.5%) | 159 (7.2%) | 307 (13.9%) | 352 (16%) | 516 (23.4%) | 761 (34.6%) | 0.298 cde |
| C4 | 2104 | 32 (1.5%) | 94 (4.5%) | 163 (7.7%) | 181 (8.6%) | 351 (16.7%) | 560 (26.6%) | 723 (34.4%) | 0.301 ef |
| C5 | 719 | 2 (0.3%) | 32 (4.5%) | 59 (8.2%) | 105 (14.6%) | 110 (15.3%) | 121 (16.8%) | 290 (40.3%) | 0.298 cde |
| C6 | 1539 | 28 (1.8%) | 52 (3.4%) | 101 (6.6%) | 136 (8.8%) | 403 (26.2%) | 417 (27.1%) | 402 (26.1%) | 0.296 cde |
| C7 | 2006 | 44 (2.2%) | 591 (29.5%) | 52 (2.6%) | 185 (9.2%) | 156 (7.8%) | 587 (29.3%) | 391 (19.5%) | 0.254 a |
| C8 | 1096 | 5 (0.5%) | 23 (2.1%) | 40 (3.6%) | 362 (33%) | 90 (8.2%) | 229 (20.9%) | 347 (31.7%) | 0.292 bc |
| C9 | 835 | 14 (1.7%) | 18 (2.2%) | 78 (9.3%) | 97 (11.6%) | 227 (27.2%) | 192 (23%) | 209 (25%) | 0.287 b |
| Sum | 24256 | 219 (0.9%) | 1391 (5.7%) | 1399 (5.8%) | 2663 (11%) | 3931 (16.2%) | 6722 (27.7%) | 7931 (32.7%) | - |

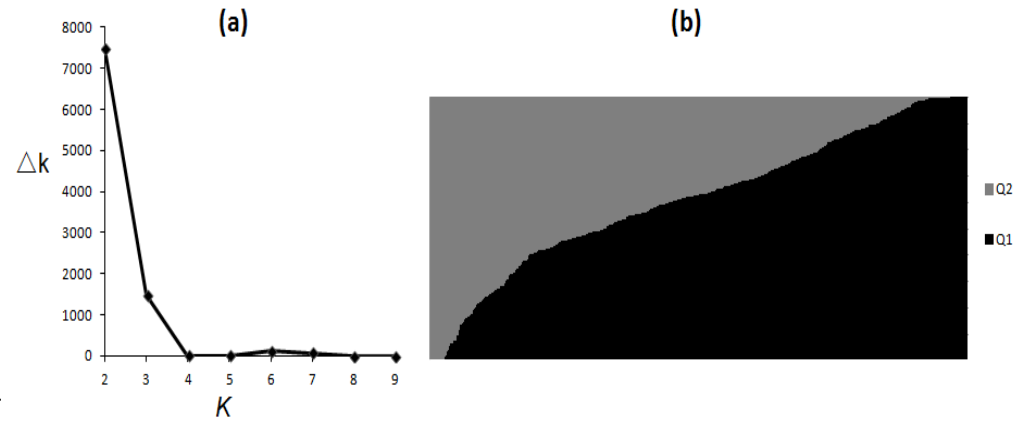
Characterization of the 472 word-wide collection



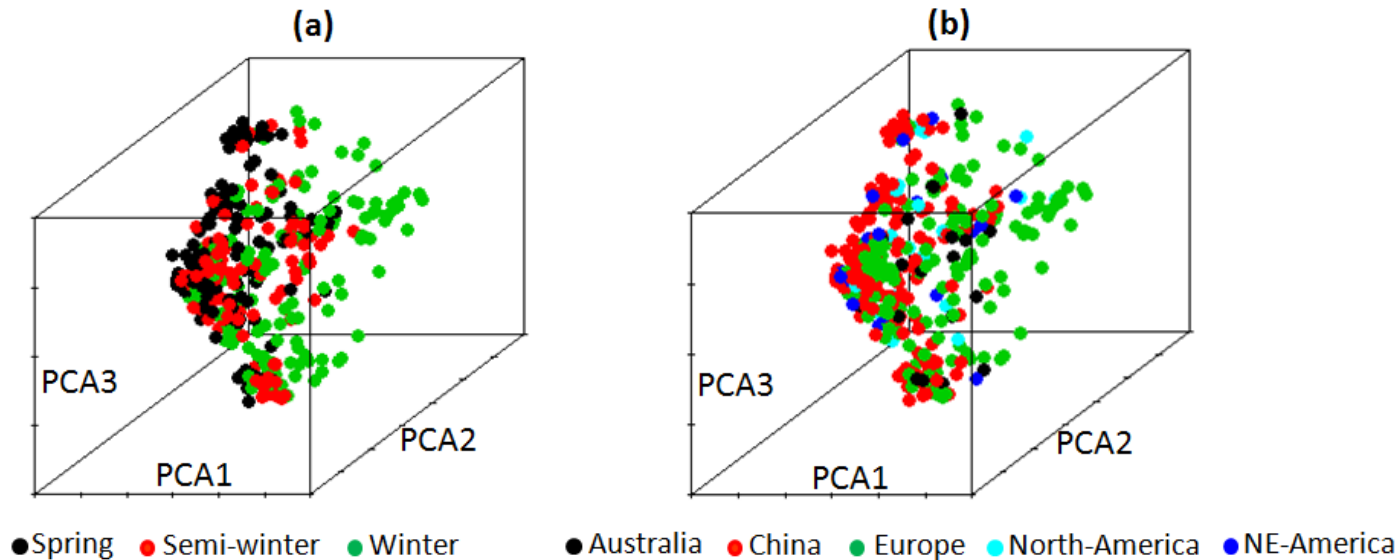
Genetic distance (Identity by state)



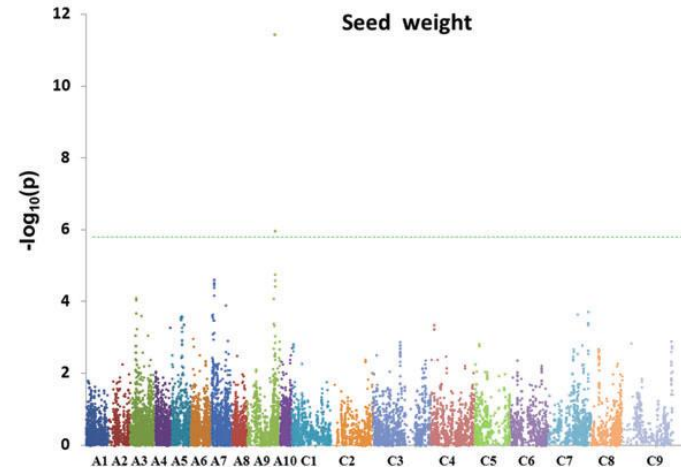
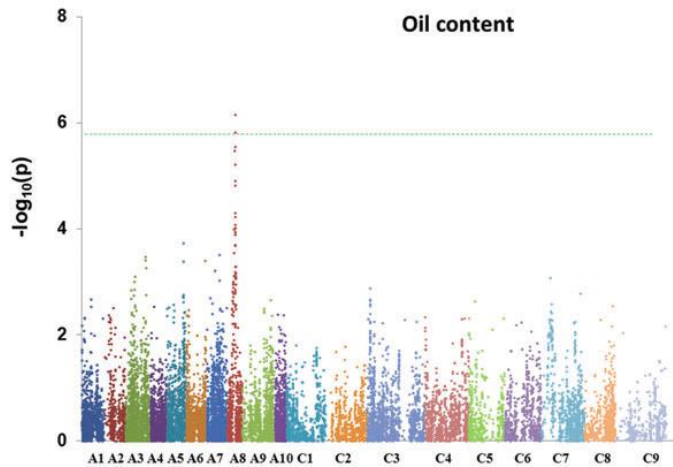
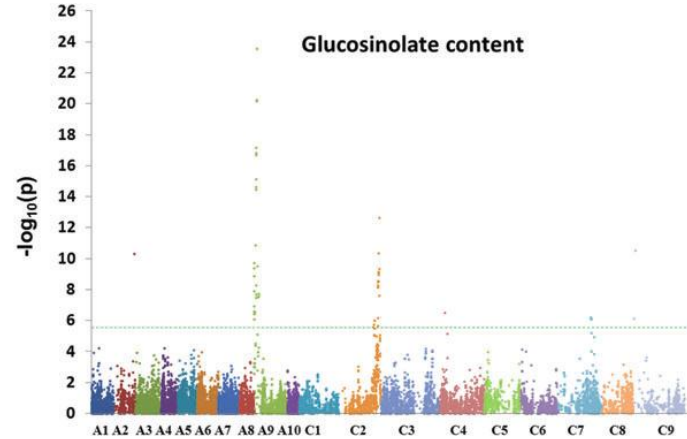
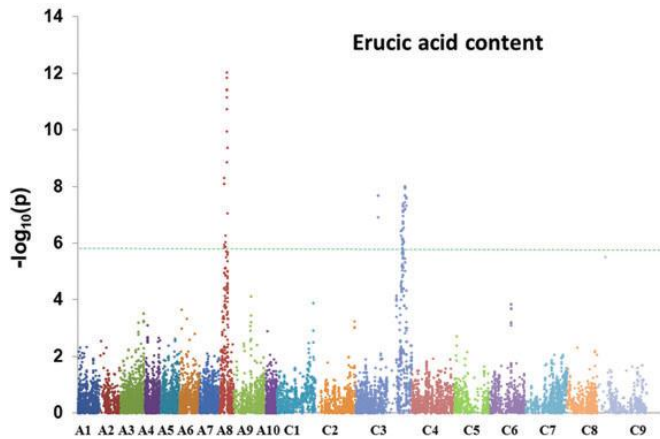
Population structure by Q matrix



Population structure by PCA



GWAS mapping in the 472 word-wide collection

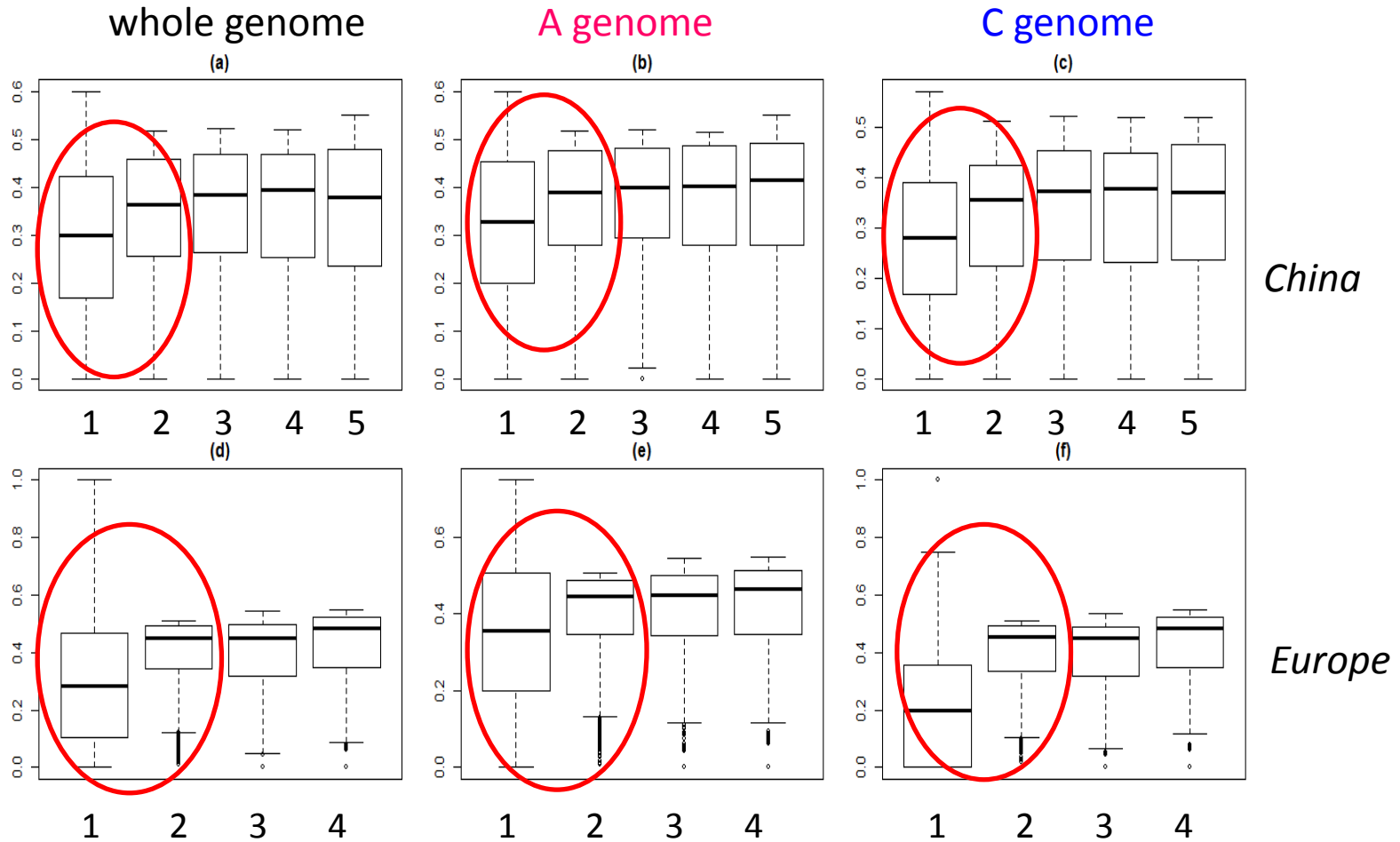


DNA RESEARCH pp. 1–13, (2014)

All these analyses indicate the rapeseed core collection and genotyping are powerful



Genetic diversity from 1950-2011 in China and Europe



1: 1950-1970 2:1971-1980 3: 1981-1990 4:1991-2000 5:2001-2010

Diversity increased sharply between 1950 to 1980 both in China and Europe



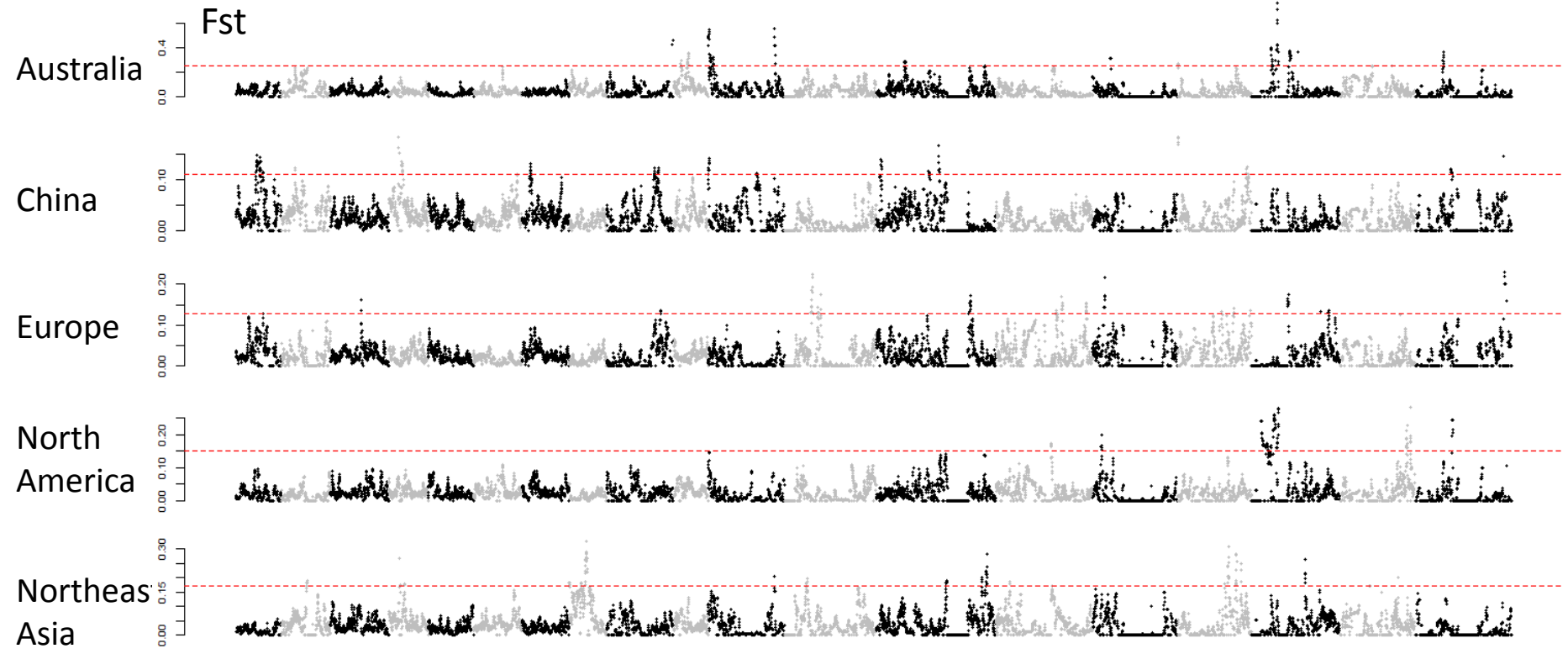
Detail summary of genetic diversity

| | China | | | Europe | | |
|-------------|---------------|---------------|--------------|---------------|---------------|--------------|
| | 1950-1970 | 1971-1980 | Increase (%) | 1950-1970 | 1971-1980 | Increase (%) |
| Genome A | 0.3135±0.1508 | 0.3618±0.1250 | 15.4 | 0.3191±0.1897 | 0.4059±0.1064 | 27.2 |
| Genome C | 0.2740±0.1454 | 0.3192±0.1345 | 16.5 | 0.2134±0.1898 | 0.4004±0.1140 | 87.6 |
| Total (A+C) | 0.2912±0.1490 | 0.3338±0.1321 | 14.6 | 0.2595±0.1969 | 0.4028±0.1108 | 55.2 |

- ✓ Genetic diversity of China < Genetic diversity of Europe
- ✓ The increase degree in A and C genome were similar in China
- ✓ Genetic diversity increased more in C genome than A genome for European groups

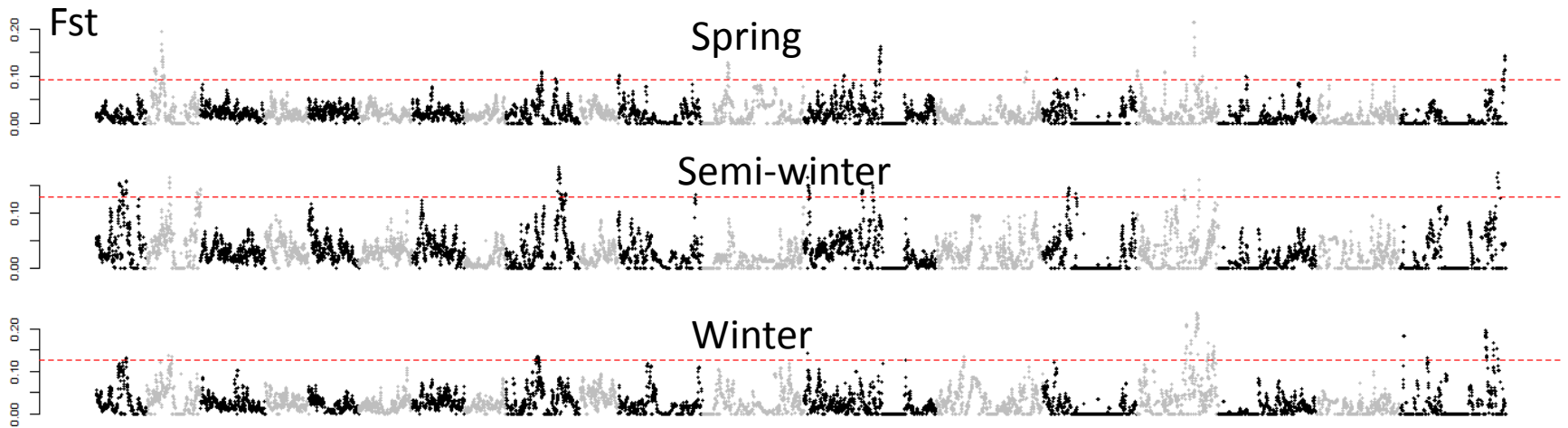


Allele preference for rapeseed collected from different regions



Skew positions indicated outliers-regions (preference regions) corresponding to special geography

Allele preference for different growth types in rapeseed collection

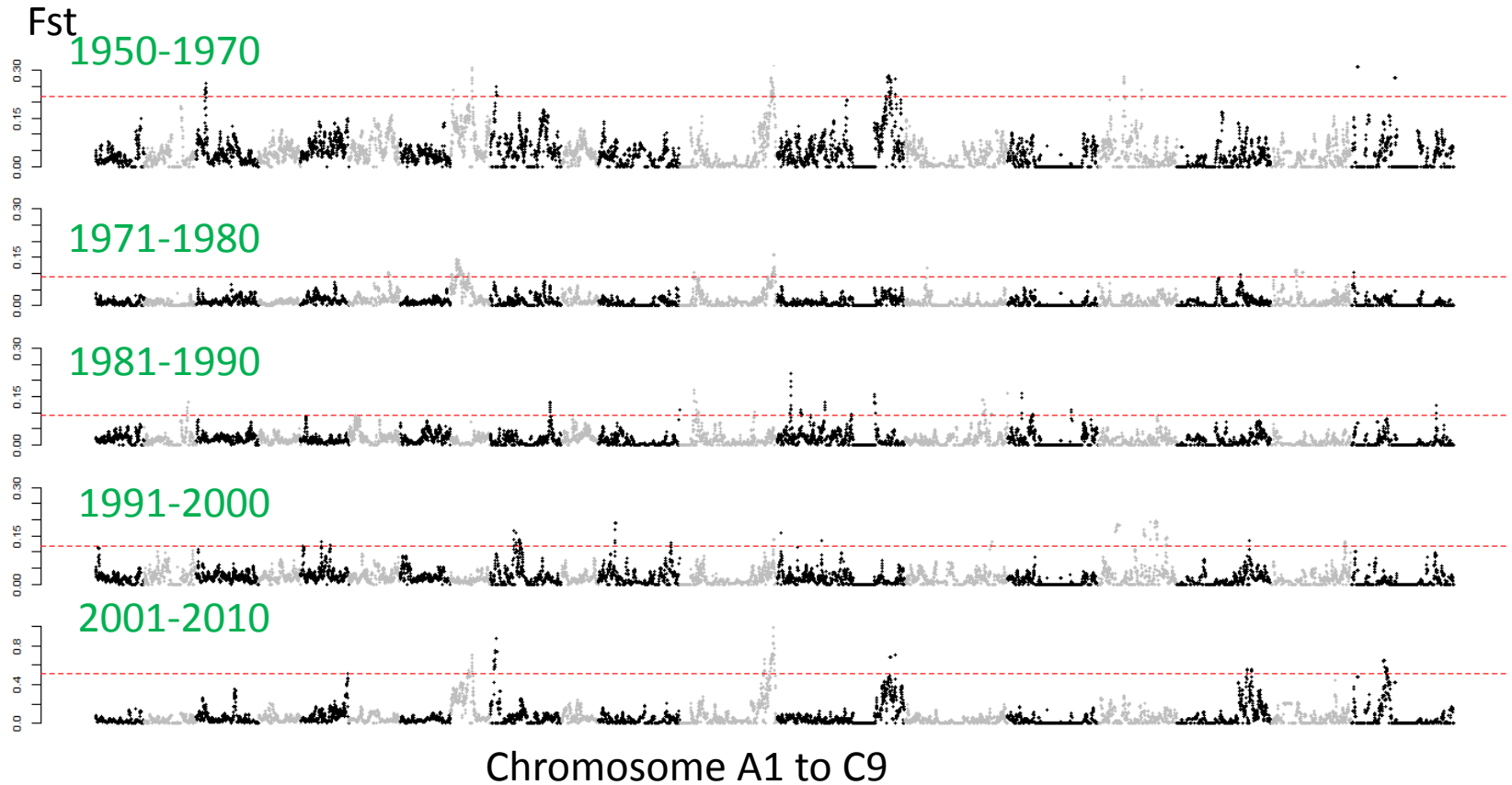


Chromosome A1 to C9

Skew positions indicated outliers-regions responding to growth types



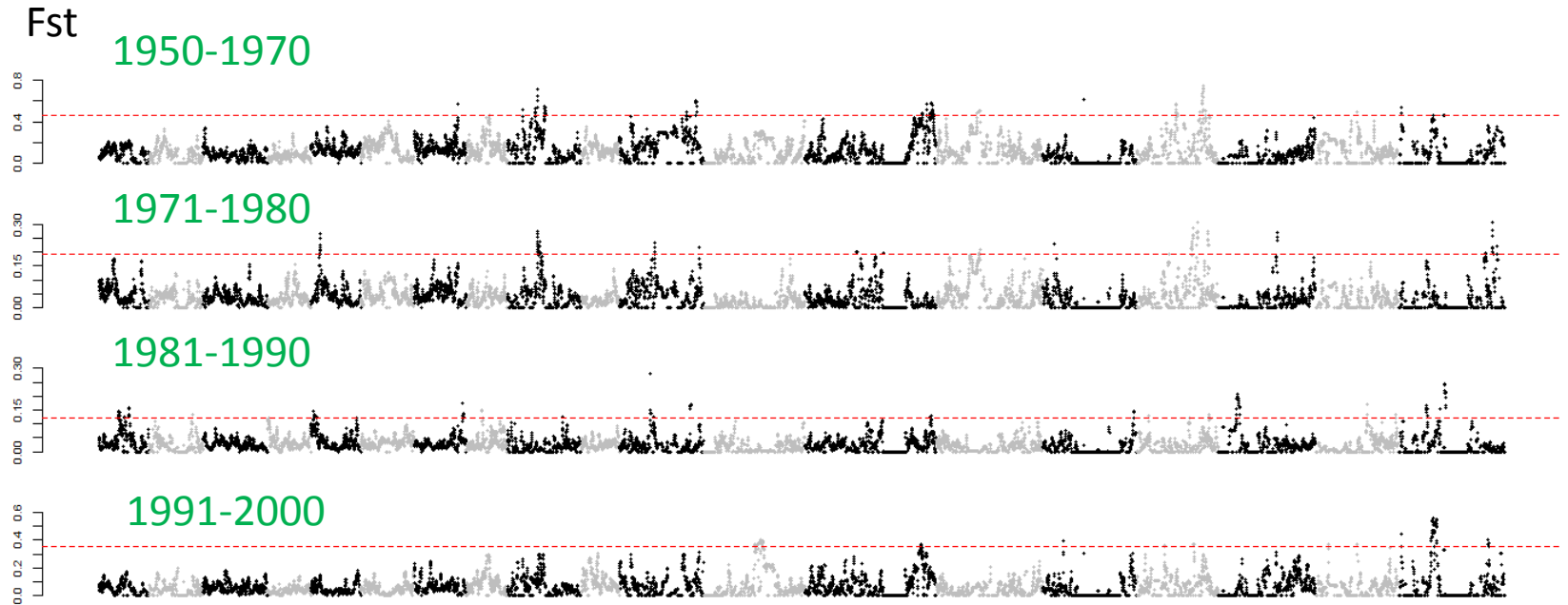
Allele preference for different release periods in Chinese rapeseed collection



Skew positions indicated outliers-regions in different breeding periods in China



Allele preference for different release period in European rapeseed collection



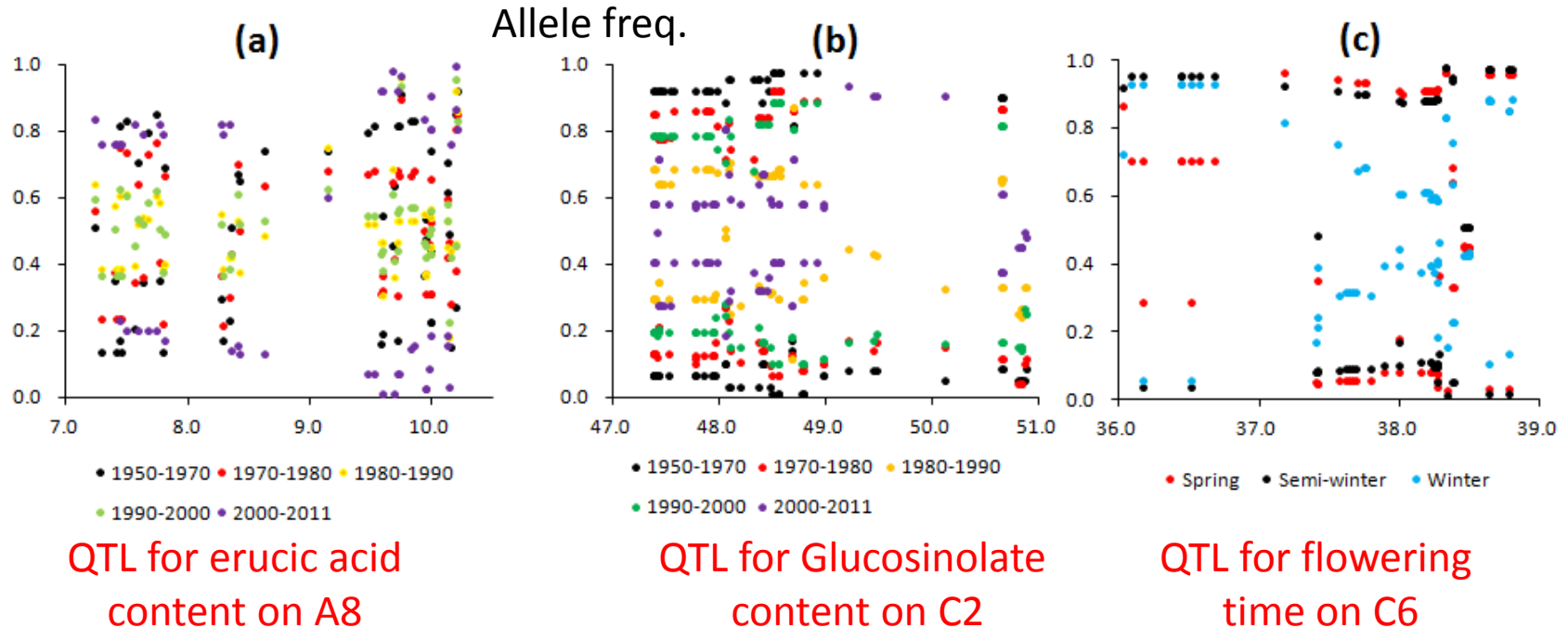
Chromosome A1 to C9

Skew positions indicated outliers-regions in different breeding periods in Europe



Allele preference loci overlapped with major QTL

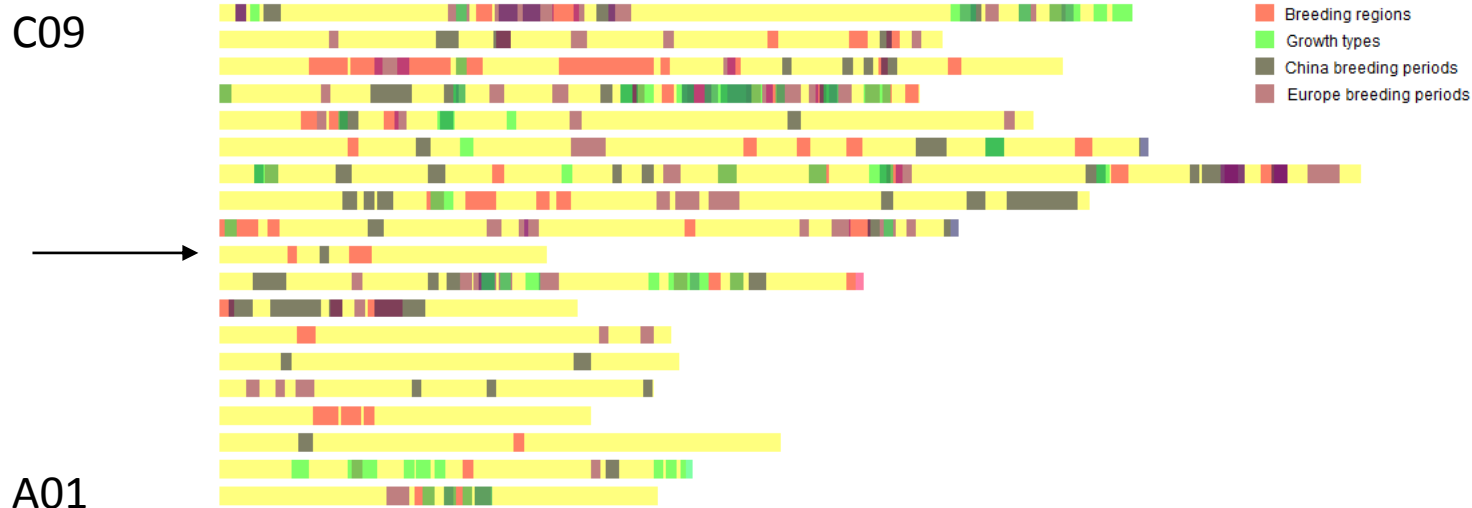
Examples to dissect the loci of allele preference



Loci of allele preference can be used for dissecting trait variations



Summary of allele preference due to breeding process



**Lots of loci subjected to be artificial selection
in rapeseed breeding programs**



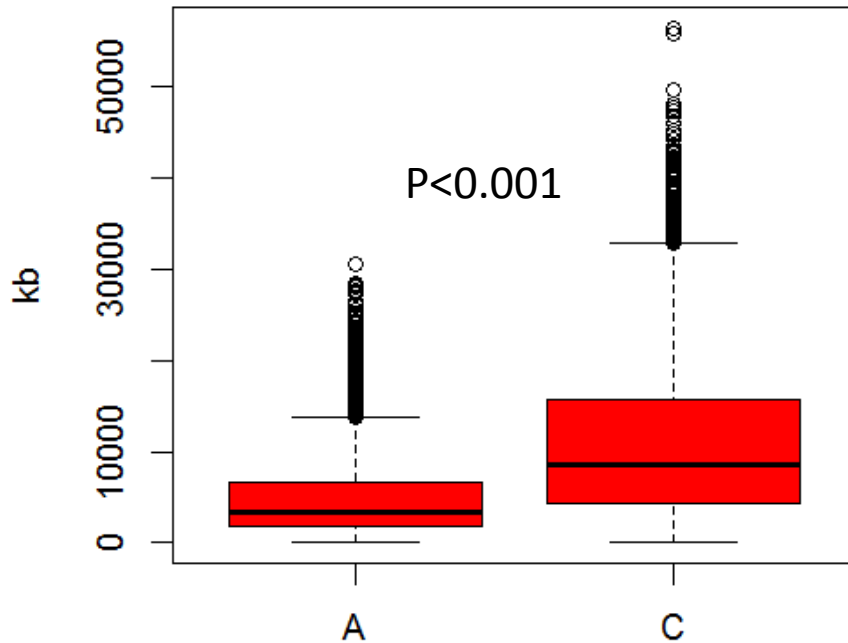
| | Total | | A genome | | C genome | |
|------------------|-----------|-------|-----------|-------|-----------|-------|
| | Size (Mb) | Count | Size (Mb) | Count | Size (Mb) | Count |
| Breeding regions | 68.35 | 70 | 17.05 | 19 | 51.3 | 51 |
| Growth types | 42.55 | 49 | 14.3 | 18 | 28.25 | 31 |
| China periods | 59.85 | 63 | 17.2 | 17 | 42.65 | 46 |
| Europe periods | 54.6 | 62 | 11.85 | 16 | 42.75 | 46 |

More and longer blocks showed selection signals in C than A genome in rapeseed

Why ?

Comparison of haplotype sharing blocks between A and C genomes

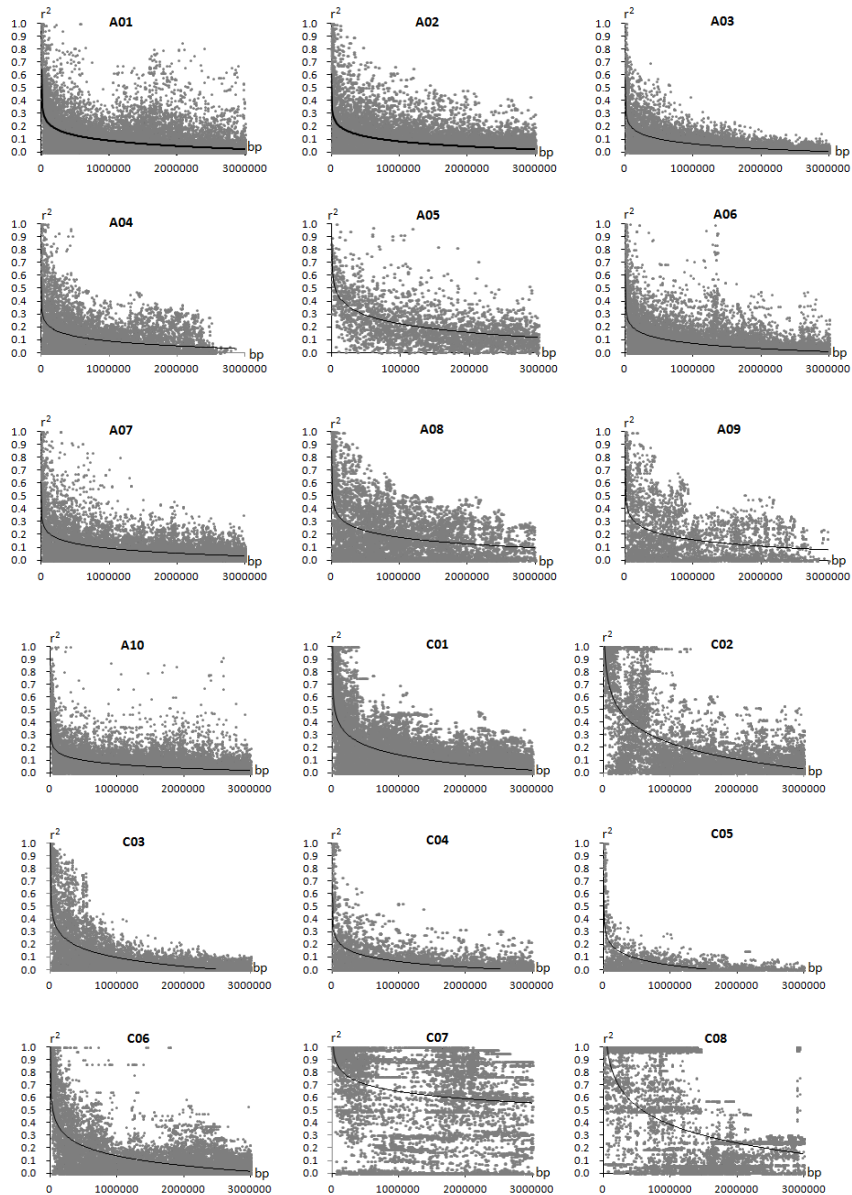
haplotype sharing block



| | Total pairs | Mean size (kb) |
|----------|-------------|----------------|
| Genome A | 12684 | 4812.7 |
| Genome C | 4964 | 11131.2 |

longer haplotype sharing blocks in C than A genomes

Longer haplotype blocks sharing produced slower LD decay in C genome



| Chr. | R2 decay to 0.1 (Mb) | R2 decay to 0.2 (Mb) |
|------|---------------------------|---------------------------|
| A01 | 0.79 | 0.15 |
| A02 | 0.65 | 0.11 |
| A03 | 0.51 | 0.08 |
| A04 | 0.81 | 0.13 |
| A05 | 0.33 | 0.12 |
| A06 | 0.64 | 0.11 |
| A07 | 0.80 | 0.13 |
| A08 | 2.58 | 0.67 |
| A09 | 2.21 | 0.55 |
| A10 | 0.48 | 0.05 |
| C01 | 1.52 | 0.61 |
| C02 | 2.06 | 1.20 |
| C03 | 1.00 | 0.37 |
| C04 | 0.52 | 0.11 |
| C05 | 0.40 | 0.11 |
| C06 | 1.37 | 0.56 |
| C07 | NA | NA |
| C08 | 3.99 | 2.48 |
| C09 | 2.02 | 1.03 |

Less recombinations in C genome in breeding programs



Summary

- ✓ We created a worldwide rapeseed core collection panel and have genotyped it
- ✓ This panel can be used for GWAS and a number of association studies were finished or are ongoing
- ✓ Genetic change were investigated for the past 60 years using this panel
- ✓ Allele preference was observed and some selected loci overlapped with previous major QTL
- ✓ Longer LD and hapotype sharing in C than A genome
- ✓ We should increase recombinations in napus C genome in rapeseed breeding programs

acknowledgement



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